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# Identification of rice sheath blight and blast quantitative trait loci in two different *O. sativa*/*O. nivara* advanced backcross populations

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**Abstract** Two accessions of *Oryza nivara*, a wild ancestral species of rice (*O. sativa*) identified as being moderately resistant to sheath blight and leaf blast disease, were used as donor parents to develop two advanced backcross populations with the US rice cultivar, Bengal, as the recurrent parent. The *O. nivara* donor parent for Wild-1 (252 BC2F1 lines) was acc. IRGC100898 and for Wild-2 (253 BC2F1 lines) was acc. IRGC104705. Both populations were genotyped with 131 simple sequence repeat markers and the linkage maps covered 1,567.5 cM (Wild-1) and 1,312.2 cM (Wild-2). Sheath blight (ShB) disease was evaluated in both inoculated greenhouse and field conditions. Days to heading (DH), plant height (PH), and plant type (PT), confounding factors for sheath blight disease under field conditions, were recorded. Leaf blast disease was rated under inoculated greenhouse conditions. Multiple interval mapping identified *qShB6* with resistance to sheath blight disease attributed to the *O. nivara* parent in the greenhouse. In the field, *qShB6* also was the most significant ShB quantitative trait locus (QTL) in all trials, with resistance attributed to *O. nivara*. In addition, *qShB1* and *qShB3* were identified in all trials but were not always attributed to the same parent. The *qShB6* QTL is in the same region as the DH-QTL, *qDH*6, and *qShB1* is in the same region as the major PH-QTL, *qPH1*, suggesting that these ShB-QTL may be confounded by other traits. Although *qShB3* did not have as large an effect as other loci, it was not confounded by either DH or PH. For leaf blast, *qBLAST8-1* was found in both populations providing resistance to races IB1 and IB49, whereas *qBLAST12* providing resistance to both races, was only found in Wild-2. Resistance was attributed to *O. nivara* for both QTL, and blast resistance genes have been previously reported in these regions.

**Keywords** *Oryza sativa* · *Rhizoctonia solani* · *Magnaporthe oryzae* · *Oryza* species · Advanced-backcross · QTL mapping · Asian rice

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## Introduction

Sheath blight (ShB) disease, caused by a soil-borne fungus *Rhizoctonia solani* Kühn, and rice leaf blast disease, caused by *Magnaporthe oryzae* B. Couch

(Couch and Kohn 2002), are the most prevalent fungal diseases of cultivated rice (*Oryza sativa* L.) and cause significant economic damage to rice production worldwide (Lee and Rush 1983; Rush and Lindberg 1996; Khush and Jena 2009; Skamnioti and Gurr 2009). Wild relatives of cultivated rice are a potential source of novel genes for insect and disease resistance, as well as tolerance to several abiotic stresses and a source of yield and yield-enhancing traits (Khush et al. 1990; Brar and Khush 1997; Jena and Khush 2000; Ali et al. 2010). Sheath blight resistant sources have been identified in *O. minuta* J.S. Presl. ex C.B. Presl. (IRGC101089) and *O. rufipogon* Griff. (IRGC100907) accessions (Amante et al. 1990). Lakshmanan (1991) reported the use of *O. officinalis* Wall ex Watt in the transfer of sheath blight resistance genes into cultivated rice through backcross breeding. Rice blast resistance genes have been identified in three wild rice relatives, including *Pi40* found in *O. australiensis* Domin (Jeung et al. 2007), *Pi9* found in *O. minuta* (Qu et al. 2006) and *Pi33* mapped in *O. rufipogon* (Berruyer et al. 2003). Evaluation of a collection of *Oryza* species accessions with US blast races revealed a few accessions that were resistant to both blast and sheath blight, including *O. nivara* Sharma et Shastry accessions IRGC100898, IRGC104443 and IRGC104705 (Eizenga et al. 2002, 2009; Prasad and Eizenga 2008).

*R. solani* is saprophytic in nature and has a very wide host range which includes rice, maize (*Zea mays* L.), soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.) and common bean (*Phaseolus vulgaris* L.) (Zhao et al. 2006b). No source of complete resistance has been identified in rice germplasm; however, some rice lines were identified as tolerant or partially resistant to sheath blight disease (Lee et al. 1999; Raina et al. 1999; Chen et al. 2000; Li et al. 2000; Meena et al. 2000; Pinson et al. 2008, Jia L et al. 2012). Sheath blight quantitative trait loci (QTL) have been identified in several recombinant inbred line or F2 populations involving two *O. sativa* cultivars having one parent as moderately resistant and the other as susceptible (summarized in Jia et al. 2009; Channamallikarjuna et al. 2010; Fu et al. 2011; Xu et al. 2011; Nelson et al. 2012). A major sheath blight QTL was identified on the bottom of chromosome 9 in several of these populations. Subsequently, this was validated using the single nucleotide polymorphism (SNP) variants identified for chromosome 9 from the whole genome sequence of 13 rice cultivars, including four resistant cultivars subjected to a principal component analysis with a biplot display (Silva et al. 2012). Most studies identifying sheath blight QTLs attributed resistance to the effect of multiple genes, and the resistance as a polygenic quantitative trait. Venu et al. (2007) identified at least 23 rice host genes involved in different biochemical pathways using DNA microarray and serial analysis of gene expression. More recently, Manosalva et al. (2009) verified different levels of resistance based on the expression of the germin-like protein (OsGLP) gene family members on rice chromosome 8. Several studies suggest that sheath blight resistance is greatly influenced by morphological traits like plant height and days to heading, as well as the relative humidity and temperature while the plants are growing (Pinson et al. 2005; Jia et al. 2009). These confounding factors, along with the quantitative nature of sheath blight resistance, make it difficult to develop resistant cultivars (Sharma et al. 2009).

For rice leaf blast, on the other hand, more than 73 resistance genes have been identified and about 17 have been cloned or fine mapped (Liu et al. 2010; Skamnioti and Gurr 2009). Many of these resistance genes map to certain regions of the rice genome, with blast gene clusters known on chromosomes 1, 2, 4, 6, 8, 9, 11 and 12. Analysis of *M. oryzae* population structure revealed that the population is composed of a limited number of lineages and predominately clonal populations (Liu et al. 2010). In tropical Asia where rice has been grown since ancient times, the number of clonal lineages is nearly seven times higher than that seen in the US populations (Correll et al. 2009; Tharreau et al. 2009).

The advanced backcross method of QTL analysis (AB-QTL) was proposed as an effective molecular breeding technique for incorporating valuable genes from exotic sources into an adapted background (Tanksley and Nelson 1996). With this method, QTL from the wild donor parents are detected by backcrossing with the adapted parent to eliminate most of the unwanted genes from the wild genetic background. The AB-QTL analysis method is a very efficient means of isolating the beneficial genes by exploiting the genetic diversity available in the exotic gene pool which is sometimes discarded due to linkage with unfavorable traits (Tanksley and McCouch 1997). In addition, the AB-QTL method can accelerate the crop improvement process because near-isogenic lines (NILs) containing the desired QTL (genes) from the wild donor in the background of the recurrent parent

can be selected from the advanced backcross population (Bernacchi et al. 1998). The value of the AB-QTL method for isolating yield and yield-enhancing alleles from *O. rufipogon* (IRGC105491) was demonstrated in rice with five different adapted cultivars being used as the recurrent parents in developing the populations (McCouch et al. 2007). Subsequently this method was used to develop populations with other *O. rufipogon* accessions (Jing et al. 2010; Tan et al. 2008; Wickneswari et al. 2012) and an *O. glumaepatula* accession (Brondani et al. 2002).

The objective of this study was to use the AB-QTL strategy to determine whether novel resistance genes could be found associated with sheath blight and blast resistance in two *O. nivara* accessions (IRGC100898; IRGC104705). In order to validate the QTL mapping results, two mapping populations were developed with a common recurrent parent, Bengal.

## Materials and methods

### Population development

The parents in the mapping populations were Bengal (PI561735; Linscombe et al. 1993), a popular southern US *tropical japonica*, medium grain rice cultivar, and two accessions of the rice progenitor species, *O. nivara* (IRGC100898; IRGC104705). These *O. nivara* accessions were collected in Orissa (IRGC100898) and Maharashtra (IRGC104705), India and were obtained from the International Rice Research Institute, Los Banos, Philippines. To develop two advanced backcross mapping populations, Bengal, which is moderately susceptible to sheath blight and susceptible to blast races IB1 and IB49 (Linscombe et al. 1993), was used as the recurrent parent, and the two *O. nivara* accessions, which were moderately resistant to sheath blight disease (Prasad and Eizenga 2008) and completely resistant to some blast races (Eizenga et al. 2009), were used as donor parents. The parents were genotyped as part of an earlier study (Eizenga et al. 2009) and these two *O. nivara* accessions were found to be closely related. Developing populations with similar backgrounds will allow the QTL to be validated across populations.

All parents in the crosses were single plant selections. Five F1 seeds were derived from the Bengal/*O. nivara* (IRGC100898) cross, henceforth identified as "Wild-1", and 14 F1 seeds from the Bengal/*O. nivara* (IRGC104705) cross, henceforth identified as "Wild-2". All F1 plants were confirmed phenotypically and backcrossed to Bengal as the male parent, resulting in 104 and 214 Wild-1 and Wild-2 BC1F1 seeds, respectively. To produce the Wild-1 BC2F1 generation, 58 BC1F1 plants were backcrossed to Bengal as the male parent and 44 plants produced at least four BC2F1 seeds. The *O. nivara* introgression was verified in all 58 Wild-1 BC1F1 plants with 24 simple sequence repeat (SSR) markers, one from each of the 12 chromosome arms. Similarly, 41 Wild-2 BC1F1 plants were backcrossed to Bengal and 39 plants produced at least four BC2F1 seeds. A total of 900 Wild-1 and 849 Wild-2 BC2F1 seeds were produced; from these, 280 seeds were selected to advance by self-pollination to the BC2F2 in the greenhouse. To ensure that each BC1F1 founder line was represented, 4–10 BC2F1 seeds were selected from each founder line. After genotyping, the BC2F2 families identified as selfs were removed, resulting in 252 Wild-1 and 253 Wild-2 BC2F2 families.

### Evaluation of sheath blight disease and agronomic traits

The 252 Wild-1 and 253 Wild-2 BC2F2 families were evaluated for reaction to sheath blight disease in the greenhouse using the microchamber method and by inoculating with *R. solani* in the field during two different seasons (BC2F2 generation in 2008 and BC2F3 progeny in 2009) at the USDA-ARS, Dale Bumpers National Rice Research Center, Stuttgart, AR, USA. The microchamber method was described by Prasad and Eizenga (2008) and Jia et al. (2007). The experiment was arranged in a randomized design with three replications, where each pot having four plants was considered a replication. Four-week-old seedlings were inoculated with a PDA agar block (7 mm diameter) containing actively growing mycelia of the *R. solani* isolate RR0140-1 at the base of the seedling. Previously, this isolate produced consistent symptoms on reference cultivars (Jia et al. 2007; Prasad and Eizenga 2008) and was confirmed as a true isolate of *R. solani* with molecular data (Wamishe et al. 2007). The inoculated pots were covered with a 2-l soft drink bottle which had the bottom and cap removed to form a microchamber. The plants were incubated for 7–10 days under greenhouse growing conditions

where the relative humidity inside the microchambers ranged from 90 to 95 % and the temperature ranged from 26 to 30 °C from day to day. The seedlings were rated for sheath blight response by measuring the culm length and disease lesion from the base of the plant. The disease index was calculated by dividing the culm length into the lesion length and multiplying by 9 as described by Jia et al. (2007).

During summer 2008, the populations were screened in the field using a randomized complete block design with two replications. Because there was limited BC2F2 seed per family, each replication had seven plants per row from each BC2F2 family with a Bengal plant at the beginning and end of the row as a border. Bengal was also used as a border row between all test rows. Plants were spaced approximately 30.5 cm apart within rows and between rows. Standard control cultivars Lemont, Cocodrie, Ahrent, Bengal, Jasmine 85 and TeQing were planted in each group of 48 rows to verify uniform disease development in the field. Inoculation and rating methods are described below.

The populations were evaluated a second year (2009) in the field using BC2F3 seed harvested in 2008 from the BC2F2 field-grown plants to be planted in 3-row plots arranged in a randomized complete block design with two replications. The three-row plots were 1.52 m long with 30.5 cm between the rows. The check cultivars Bengal, Lemont, Cocodrie, TeQing and Jasmine85 were planted in each group of 35 BC2F1 progeny lines.

The field plots were inoculated with a mixture of *R. solani* mycelia growing on a media of crushed maize and ryegrass (*Lolium perenne* L.) (F. N. Lee, University of Arkansas, unpublished method). Inoculum of the isolate RR0140-1 was spread at the base of each plant, with 30 g of inoculum spread over each progeny row. The progeny rows were rated for sheath blight reaction approximately 6 weeks after inoculation on a 0 (no disease) to 9 (nearly dead) scale (Marchetti and Bollich 1991) with each unit of the scale representing the percentage of culm length above the water line that had disease lesions.

Three agronomic traits—days to heading, plant height and plant type (tiller angle)—were collected from the field experiment. Days to heading was calculated as the number of days required to have 50 % of the plants within a row with at least one tiller at anthesis. At maturity, plant height (cm) was measured from the base of the plant to the tip of the tallest panicle excluding the awn from three plants in each row. Plant type was estimated by visual observation from three plants in a row at the milky dough stage on a 1–9 scale with 1 (erect), 3 (intermediate), 5 (open), 7 (spreading) and 9 (procumbent) (IRRI 2002). Correlation coefficients between the traits were estimated using the trait analysis feature within the software QGene 4.3.9 (Joehanes and Nelson 2008).

## Evaluation of blast disease

Pathogenicity tests of *M. oryzae* were conducted on BC2F3 seedlings based on seed availability, with two replications for Wild-1 and four replications for Wild-2. The cultivars Bengal, Early, Kaybonnet, Katy, LaGrue, Maybelle, Newbonnet, M-201 and Rosemont were used as controls. Seedlings were inoculated with *M. oryzae* races, IB1 and IB49, which are common in the USA (Atkins et al. 1967). The fungal spores were grown as described by Jia and Liu (2011). Six seeds were planted in soil, in 4.0 × 6.0 cm pots, which were placed in trays and watered as needed. When the seedlings were at the 3–4-leaf stage, they were drought-stressed to enhance the blast disease reaction, and a suspension of the specific *M. oryzae* race spores (100,000–200,000 spores per ml) was applied to the leaves with an airbrush. The trays with the seedlings were placed in a large plastic bag which was closed at the top to maintain 70 % humidity and left on the laboratory bench overnight. The next morning, the bags were removed and the seedlings placed in the greenhouse using supplemental light as needed, depending on the amount of natural sunlight; they were watered with a 20–20–20 liquid fertilizer solution, and rated approximately 1 week later for blast symptoms on a 0 (no disease) to 9 (dead) rating scale.

## Molecular marker analysis

Leaf tissue was harvested from the individual BC2F1 plants and two parents for each population grown in the greenhouse. DNA was extracted using the method described by Tai and Tanksley (1990) and genotyped with 131 SSR markers (marker sequence and genomic location information available from Gramene, http://www.gramene.org). PCR amplification and

analysis of the SSR markers was performed as described in Costanzo et al. (2011).

Segregation of marker loci was evaluated with a Chi-squared test. The expected genotypic frequency for each marker locus was 0.75 for BC2 families homozygous for the Bengal allele and 0.25 per locus for heterozygous BC2 families.

### Percentage of the *O. nivara* genome in the BC2F1 plants

The percentage of the *O. nivara* genome introgressed into each BC2F1 plant was determined by the GGT: Graphical Genotype software (Van Berloo 2008). The interval between two consecutive marker loci was designated towards one parent if those two loci had marker alleles from the same parent. On the other hand, if one marker allele originated from the *O. nivara* parent and the other marker allele from the Bengal parent, then half of the interval was designated towards each parent.

### Linkage mapping and QTL analysis

The linkage map for the population was created using JoinMap 4.0 software (Van Ooijen 2006). The marker loci were assigned to linkage groups with the program default setting. Genetic distances between the loci were expressed in centimorgans using the Kosambi mapping function (Kosambi 1944). The fixed order command was used to identify the best probable marker order within each chromosome and this order was based on the marker's position on the Nipponbare 2009 genome (available at http://www.gramene.org). For markers not already positioned on the 2009 genome, we BLASTed the primers to determine the most probable physical location for the marker.

QTL were identified using multiple interval mapping (MIM) performed by QGene 4.3.10 (Joehanes and Nelson 2008). The scan interval was set at 2 cM using the Kosambi mapping function with an automatic selection of peak markers for putative QTL as cofactor. The LOD thresholds for declaring the presence of significant QTL for each trait and study were determined using QGene to run 1,000 permutations and $\alpha = 0.05$. The additive effect of each QTL and its percentage of phenotypic variance explained were also calculated.

## Results

### Marker segregation

The 131 SSR markers were distributed throughout the 12 rice chromosomes for a total length of 1,567.5 cM with an average interval of 11.97 cM between two markers in Wild-1, and 1,312.2 cM with an average interval of 10.02 cM between two markers for Wild-2. The marker order within the individual chromosomes was identical to their order based on physical position on the Nipponbare genome (http://www.gramene.org/markers/). During population development in the greenhouse, the only intentional selection imposed was against extreme sterility by excluding the most sterile BC1, and thus the expected genotypic ratio in the BC2 generation was 3:1 for homozygous Bengal alleles:heterozygous (Bengal/*O. nivara*) per locus. In the Wild-1 population 18 (13.7 %) of the 131 markers were skewed towards the recurrent parent Bengal and 22 (16.8 %) markers were skewed towards heterozygosity ($\chi^2 > 6.6$, $P < 0.01$). The most distorted regions, those with four or more skewed markers being closely linked, were detected on chromosome 6 which was skewed toward the Bengal parent alleles and chromosome 8 which was skewed toward heterozygosity (Supplementary Fig. 1a). In the Wild-2 population 35 (26.7 %) of the 131 markers were skewed towards the recurrent parent Bengal and 16 (12.28 %) markers were skewed towards the Bengal/*O. nivara* class ($\chi^2 > 6.4, P < 0.01$). The most distorted regions, with at least four closely linked markers, were located on chromosomes 3 (bottom), 6 and 10 which were skewed toward the Bengal parent and the middle of chromosome 3 which was skewed toward heterozygosity (Supplementary Fig. 1b). In both populations, the markers in the middle of chromosome 6, RM3431, RM3183 and RM541, and at the end, RM343 and RM340, were significantly skewed toward an excess of Bengal alleles.

### Percentage of the *O. nivara* genome in the BC2F1 plants

Marker analysis revealed the heterozygous *O. nivara* genome segments in the Wild-1 BC2F1 plants varied from 8.5 to 63.5 % with an average proportion of 25.0 % and in the Wild-2 BC2F1 plants from 5.3 to 43.8 % with an average proportion of 23.1 %. These

genomic proportions fit the expected genotypic ratio of 75 % recurrent parent and 25 % heterozygous donor parent in the BC2 generation. These observed and expected ratios confirm there was no serious selection in favor of one parent over the other. The average number of recombinations over all the BC2F1 progeny lines was 18.3 in Wild-1 and 14.3 in Wild-2. For each chromosome, the heterozygous regions ranged from single marker introgressions to whole chromosomes across the BC2F1 progeny lines.

## Phenotypic distribution

Transgressive variation was observed for all of the traits in one population or the other (Table 1). The standard check cultivars included with both sheath blight evaluation methods verified there was sufficient disease pressure in each of the present studies for testing the sheath blight reaction of the population (data not shown). Sheath blight ratings from both the greenhouse and field showed continuous distributions (Fig. 1). Overall, Wild-2 was slightly more susceptible than Wild-1 and the field ratings using BC2F3 progeny in 2009 were skewed toward the more susceptible parent with a mean of 5.4 for Wild-1 and 5.9 for Wild-2. The more susceptible rating overall in 2009 may reflect the increased homozygosity for the susceptible alleles in the BC2F3 as compared to the BC2F2 generation; thus the disease is more apparent and the ratings are more toward susceptibility. The *O. nivara* parent is weedy, and thus it was not included in the field trials.

The distributions for days to heading (Supplementary Fig. 2A) were skewed toward the Bengal parent in both Wild-1 and Wild-2. In both populations, the distributions were more skewed toward Bengal among the BC2F3 progeny plants in 2009 compared to the BC2F2 progeny plants in 2008. On the other hand, the distributions for plant height (Supplementary Fig. 2B) and plant type or tiller angle (Supplementary Fig. 2C) were more skewed toward the Bengal parent in the BC2F2 progeny plants in 2008 than in the BC2F3 progeny plants in 2009. Also, Wild-2 was more skewed for plant height in the BC2F3 generation than Wild-1. Data from the *O. nivara* parents grown in the greenhouse revealed they were photoperiod-sensitive, and thus the days to heading and plant height varied depending on the planting date. The plant type was "5" for both *O. nivara* parents regardless of planting date.

**Table 1** Summary statistics of the traits measured on parents and Wild-1 with 252 progeny lines and Wild-2 with 253 progeny lines

| Traits | Progeny | | | Parents | |
|---|---|---|---|---|---|
| | Mean | Range | SD | Bengal | *O. nivara*[a] |
| *Wild 1 population* | | | | | |
| ShB score (microchamber) | 5.2 | 2–9 | 1.4 | 6.2 | 3.4 |
| ShB score (2008 field) | 5.1 | 2–9 | 1.9 | 6.5 | – |
| ShB score (2009 field) | 5.4 | 1–8 | 1.3 | 5.8 | – |
| Blast race IB1 | 6.9 | 0–9 | 2.8 | 9.0 | 0 |
| Blast race IB49 | 6.5 | 0–9 | 2.0 | 8.5 | 0 |
| Days to heading 2008 | 85.0 | 73–113 | 7.1 | 82.0 | – |
| Days to heading 2009 | 82.2 | 78–91 | 1.5 | 79.8 | – |
| Plant height (cm) 2008 | 98.1 | 71–138 | 13.6 | 95.0 | – |
| Plant height (cm) 2009 | 113.4 | 80–180 | 7.3 | 96.3 | – |
| Plant type 2008 | 2.3 | 1–8 | 1.9 | 1.0 | – |
| Plant type 2009 | 2.8 | 1–7 | 0.8 | 1.2 | – |
| *Wild 2 population* | | | | | |
| ShB score (microchamber) | 5.4 | 2–9 | 1.3 | 6.3 | 2.7 |
| ShB score (2008 field) | 5.5 | 2–9 | 2.0 | 6.5 | – |
| ShB score (2009 field) | 5.9 | 1–9 | 1.5 | 6.4 | – |
| Blast race IB1 | 4.8 | 0–9 | 2.2 | 7.3 | 0 |
| Blast race IB49 | 5.5 | 0–9 | 1.9 | 6.5 | 0 |
| Days to heading 2008 | 91.4 | 76–119 | 8.8 | 82.3 | – |
| Days to heading 2009 | 84.3 | 77–96 | 4.8 | 82.0 | – |
| Plant height (cm) 2008 | 102.8 | 68–153 | 16.3 | 94.8 | – |
| Plant height (cm) 2009 | 113.5 | 80–190 | 23.6 | 92.8 | – |
| Plant type 2008 | 1.7 | 1–9 | 1.5 | 1.1 | – |
| Plant type 2009 | 2.6 | 1–7 | 1.4 | 1.4 | – |

The sheath blight tests in the microchamber and field testing in 2008 were conducted on BC2F2 and the blast tests and field testing in 2009 were conducted on BC2F3 progeny

*SD* standard deviation, *ShB* sheath blight

[a] Both *O. nivara* parents are photoperiod-sensitive and weedy, and therefore were not grown in the field. Data from greenhouse-grown plants showed that days to heading and plant height varied depending on the planting date. Those planted in October flowered in about 93 days with a plant height of 61 cm compared to 100 days and 110 cm tall for those planted in January. The plant type was "5" for both *O. nivara* parents regardless of planting date

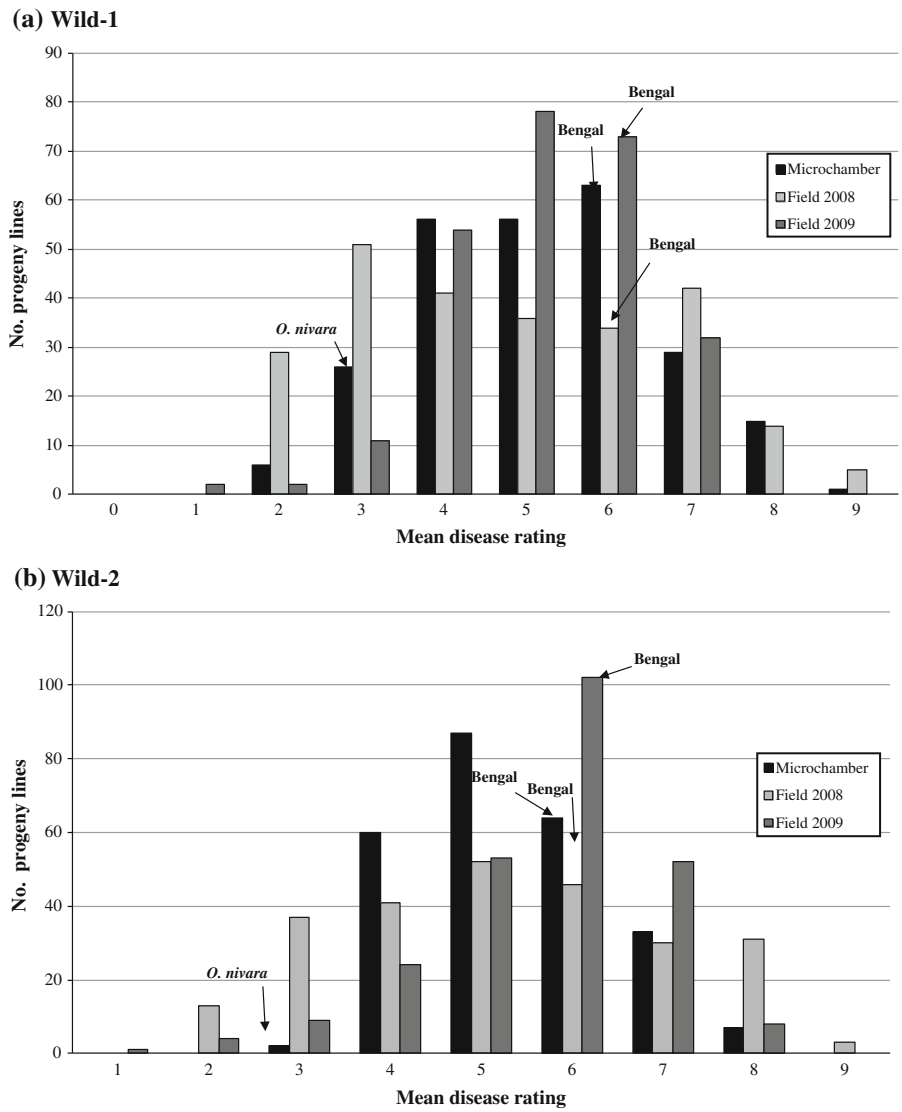


**Fig. 1** Distribution of disease ratings on a "0" (no disease) to "9" (dead) scale for **a** 252 Wild-1 and **b** 253 Wild-2 progeny lines rated for reaction to sheath blight disease using the microchamber method in the greenhouse and after field inoculation with *R. solani* in 2008 with BC2F2 progeny lines and in 2009 using BC2F3 lines. The *O. nivara* parents were only rated using the microchamber method because they are weedy and cannot be planted in the field

The blast ratings for the standard check cultivars included with each replication verified sufficient disease pressure. The blast ratings of the BC2F3 progeny (Fig. 2) for both blast races IB1 and IB49 were skewed toward the susceptible parent, with Wild-1 showing more susceptibility than Wild-2. Interestingly, Wild-1 showed more susceptibility to blast race IB1 whereas Wild-2 was more susceptible to IB49.

## Trait relationship

Correlation coefficients (Table 2) revealed that sheath blight disease development in the field had a very significant negative correlation with both days to heading and plant height, with the days to heading correlations being more significant than plant height in both populations for both years. The correlations between sheath blight disease and plant type were weak at best in both populations over both years. Sheath blight disease development in the microchamber was more significantly correlated with field sheath blight ratings in 2008 than in 2009 in both populations. This is probably due to the populations being the BC2F2 generation for both the microchamber and 2008 field testing, whereas the 2009 field testing was conducted on the BC2F3 plants. All traits collected in the field—sheath blight disease, days to heading, plant height and plant type—were significantly positively

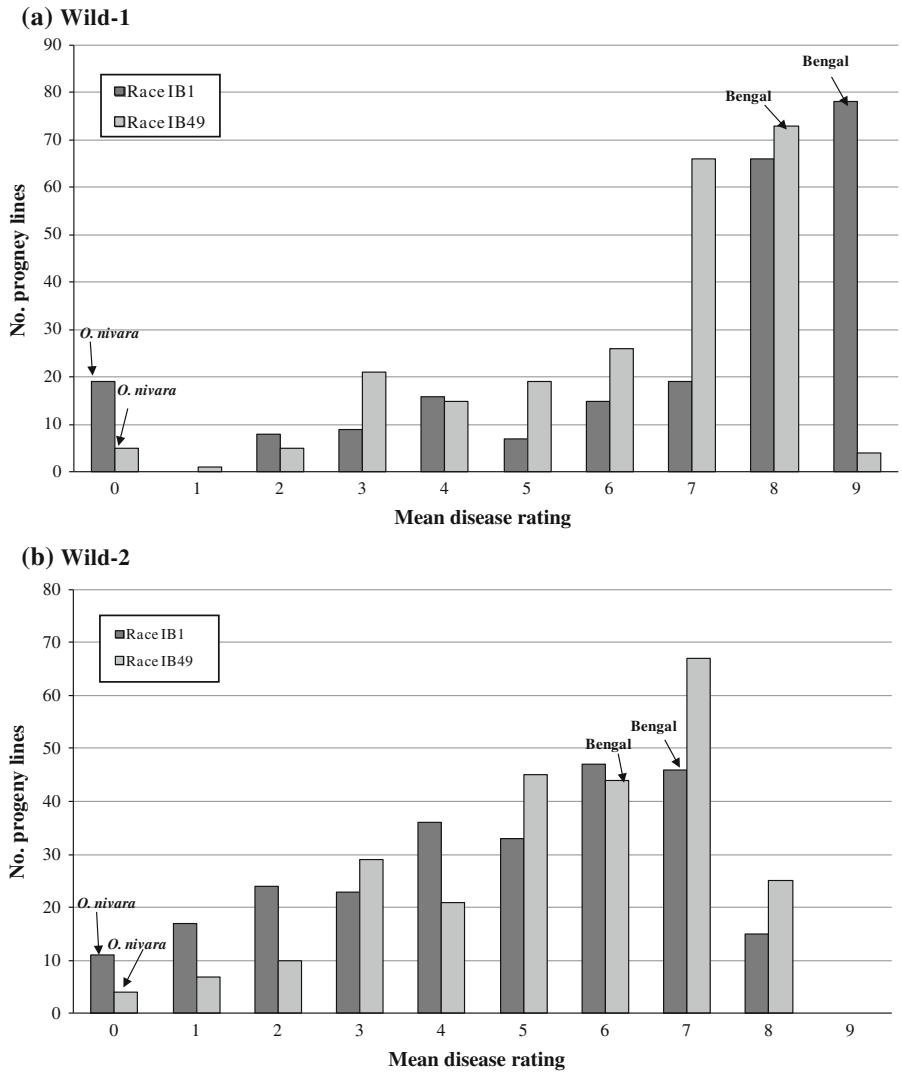


**Fig. 2** Distribution of the disease ratings on a "0" (no disease) to "9" (dead) scale for **a** 252 Wild-1 and **b** 253 Wild-2 BC2F3 progeny lines rated for reaction to leaf blast races IB1 and IB49

correlated across years in both populations, with plant height across years having the most significant correlation. In both populations, the blast ratings for the two races were significantly correlated with each other. No other correlations with blast disease were noted for the other traits measured in this population.

## QTL for sheath blight resistance

The sheath blight disease testing with the microchamber method revealed four ShB-QTL with LOD scores greater than the permuted LOD threshold across both populations (Table 3; Fig. 3). Only *qShB-mc6* found in Wild-1 was attributed to the *O. nivara* parent. The remaining ShB-QTL, *qShB-mc5*, *qShB-mc7* and *qShB-mc12*, were significant in Wild-2 and attributed to Bengal. In addition, a subthreshold LOD peak was observed near *qShB-mc5* in Wild-1.

The sheath blight disease testing in the field revealed five ShB-QTL that had LOD scores above the permuted LOD thresholds ($\alpha = 0.05$) in at least one trial. As for the microchamber evaluations, the most significant ShB-QTL was again *qShB6* with the resistance attributed to the *O. nivara* parent and identified across both populations and both years. With *qShB6* mapping to a skewed genomic region, the estimated location and additive effects (Table 3) are less precise, but the regularity with which this genomic region was associated with sheath blight resistance in both field and microchamber studies

**Table 2** Pearson's correlation coefficients between the traits measured on the BC2F2 progenies derived from the two Bengal/*O. nivara* backcross populations, Wild-1 and Wild-2

| Wild-2 | Wild-1 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | ShB reaction (MC) | ShB reaction (2008 field) | ShB reaction (2009 field) | Days to heading (2008) | Days to heading (2009) | Plant height (2008) | Plant height (2009) | Plant type (2008) | Plant type (2009) | Blast race IB1 | Blast race IB49 |
| ShB reaction (MC) | | 0.45*** | 0.15* | −0.25*** | −0.16* | −0.06 | −0.04 | 0.14** | 0.12 | −0.10 | −0.14* |
| ShB reaction (2008 field) | 0.46*** | | 0.38*** | −0.46*** | −0.32*** | −0.22*** | −0.18** | 0.10 | 0.03 | −0.08 | 0.01 |
| ShB reaction (2009 field) | 0.15* | 0.37*** | | −0.34*** | −0.43*** | −0.20** | −0.25*** | 0.03 | −0.04 | −0.02 | 0.10 |
| Days to heading (2008) | −0.08 | −0.38*** | −0.57*** | | 0.50*** | −0.05 | 0.02 | 0.05 | 0.06 | 0.02 | 0.02 |
| Days to heading (2009) | −0.08 | −0.23*** | −0.46*** | 0.41*** | | 0.03 | −0.02 | 0.19** | 0.13* | 0.02 | 0.06 |
| Plant height (2008) | −0.25*** | −0.28*** | 0.06 | −0.14* | −0.14* | | 0.76*** | −0.15* | 0.17** | 0.00 | −0.07 |
| Plant height (2009) | −0.21*** | −0.26*** | 0.19** | −0.29*** | −0.25*** | 0.82*** | | −0.05 | 0.35*** | 0.01 | −0.08 |
| Plant type (2008) | 0.02 | −0.04 | −0.01 | 0.03 | 0.25*** | −0.19** | −0.05 | | 0.62*** | 0.00 | −0.01 |
| Plant type (2009) | −0.03 | −0.14* | 0.10 | −0.08 | 0.02 | 0.31*** | 0.40*** | 0.47*** | | −0.08 | −0.10 |
| Blast race IB1 | 0.09 | 0.18** | −0.14 | −0.12 | −0.05 | −0.14 | −0.05 | −0.03 | −0.11 | | 0.56*** |
| Blast race IB49 | −0.09 | 0.00 | −0.07 | 0.01 | 0.03 | −0.08 | 0.00 | −0.07 | −0.12 | 0.66*** | |

Correlations for the Wild-1 population are given above the diagonal and those for the Wild-2 population are listed below the diagonal

*ShB* sheath blight, *MC* microchamber

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

**Table 3** List of the QTLs for leaf blast disease reaction for races IB1 and IB49; sheath blight disease reaction in the greenhouse and field for two different years; days to heading, plant height and plant type (tiller angle), confounding factors for sheath blight disease in the field for 2 years

| QTL[a] | Chr. | Wild-1 population | | | | | Wild-2 population | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Marker interval | Peak marker | LOD peak | Additive effect[b] | PVE[c] | Marker interval | Peak marker | LOD peak | Additive effect[b] | PVE[c] |
| *Sheath blight microchamber in the greenhouse* | | | | | | | | | | | |
| *qShB5-mc* | 5 | *RM122–RM5796* | *RM5796* | *1.6* | *–0.38* | *3.0* | *RM122–RM413* | *RM5796* | *3.1* | *–0.02* | 5.4 |
| *qShB6-mc* | 6 | RM3183–RM541 | RM3183 | 3.3 | 0.24 | 5.8 | | | | | |
| *qShB7-mc* | 7 | | | | | | RM295–RM5711 | RM5711 | 3.2 | –0.38 | 5.6 |
| *qShB12-mc* | 12 | | | | | | RM5746–RM277 | RM5746 | 3.0 | –0.62 | 5.3 |
| *Sheath blight—field 2008* | | | | | | | | | | | |
| *qShB1* | 1 | *RM1361–RM104* | *RM104* | *1.9* | *–0.91* | *3.4* | *RM431–RM1361* | *RM1361* | *4.7* | *0.31* | 8.2 |
| *qShB3* | 3 | *RM232–RM282* | *RM232* | *2.1* | *–0.38* | *3.7* | *RM232–RM282* | *RM232* | *5.4* | *0.30* | 9.3 |
| *qShB6* | 6 | *RM3431–RM3183* | *RM3183* | *4.7* | *0.39* | *8.2* | *RM253–RM3431* | *RM3431* | *11.1* | *0.69* | 18.2 |
| *qShB7* | 7 | | | | | | RM295–RM5711 | RM295 | 3.7 | –0.20 | 6.5 |
| *qShB11* | 11 | | | | | | RM332–RM21 | RM536 | 4.2 | –0.40 | 7.4 |
| *Sheath blight—field 2009* | | | | | | | | | | | |
| *qShB1* | 1 | *RM1361–RM104* | *RM1361* | *2.3* | *–0.02* | *4.0* | *RM403–RM431* | *RM315* | *1.6* | *–0.18* | *3.0* |
| *qShB3* | 3 | *RM232–RM282* | *RM232* | *1.4* | *–0.19* | *2.5* | *RM232–RM282* | *RM232* | *1.9* | *0.80* | *3.5* |
| *qShB6* | 6 | *RM3431–RM3183* | *RM3431* | *7.8* | *0.03* | *13.3* | *RM253–RM3431* | *RM3431* | *21.2* | *0.43* | *32.0* |
| *qShB7* | 7 | | | | | | *RM5711–RM2* | *Rid12* | *2.4* | *0.15* | *4.3* |
| *Days to heading—field 2008* | | | | | | | | | | | |
| *qDH3* | 3 | *RM22–RM545* | *RM22* | *4.6* | *–0.95* | *8.0* | *RM22–RM545* | *RM22* | *11.0* | *–1.19* | *18.2* |
| *qDH6* | 6 | *RM3431–RM3183* | *RM3431* | *26.2* | *–2.80* | *38.0* | *RM3431–RM3183* | *RM3431* | *34.1* | *–3.01* | *46.2* |
| *Days to heading—field 2009* | | | | | | | | | | | |
| *qDH3* | 3 | *RM22–RM545* | *RM22* | *21.5* | *–0.34* | *32.4* | *RM22–RM545* | *RM22* | *42.6* | *–0.34* | *53.9* |
| *qDH4* | 4 | RM451–RM255 | RM255 | 21.7 | –3.09 | 32.8 | | | | | |
| *qDH6* | 6 | *RM3431–RM541* | *RM541* | *20.0* | *–3.17* | *30.7* | *RM253–RM7551* | *RM541* | *30.3* | *–4.67* | *42.3* |
| *qDH8* | 8 | RM1148–RM72 | RM72 | 21.2 | –3.04 | 32.1 | | | | | |

**Table 3** continued

| QTL[a] | Chr. | Wild-1 population | | | | | Wild-2 population | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Marker interval | Peak marker | LOD peak | Additive effect[b] | PVE[c] | Marker interval | Peak marker | LOD peak | Additive effect[b] | PVE[c] |
| *Plant height—field 2008* | | | | | | | | | | | |
| *qPH1* | 1 | *RM315–RM431* | *RM431* | *17.3* | *–2.44* | *27.0* | *RM315–RM431* | *RM431* | *50.5* | *–3.10* | *60.1* |
| *qPH12* | 12 | | | | | | RM519–RM270 | RM519 | 8.3 | –2.06 | 14.0 |
| *Plant height—field 2009* | | | | | | | | | | | |
| *qPH1* | 1 | *RM431–RM1361* | *RM431* | *31.6* | *–2.46* | *43.9* | *RM315–RM431* | *RM431* | *81.4* | *–4.63* | *77.3* |
| *qPH12* | 12 | | | | | | *RM277–RM519* | *RM519* | *3.6* | *–1.14* | *6.3* |
| *Plant type—field 2008* | | | | | | | | | | | |
| *qPT9* | 9 | *RM107–RM215* | *RM215* | *66.8* | *–0.24* | *70.5* | *RM409–RM215* | *RM107* | *43.5* | *–0.08* | *54.7* |
| *Plant type—field 2009* | | | | | | | | | | | |
| *qPT1* | 1 | *RM1361–RM104* | *RM104* | *5.1* | *–0.16* | *9.0* | *RM431–RM1361* | *RM1361* | *10.4* | *–0.25* | *17.2* |
| *qPT9* | 9 | *RM107–RM215* | *RM107* | *32.5* | *0.04* | *44.8* | *RM107–RM215* | *RM215* | *15.4* | *0.01* | *24.4* |
| *Leaf blast race IB1* | | | | | | | | | | | |
| *qBLAST8* | 8 | *RM1148–RM210* | *RM72* | *40.7* | *0.46* | *52.5* | *RM1148–RM23000* | *RM72* | *2.5* | *0.08* | *4.5* |
| *qBLAST8* | 8 | | | | | | RM210–RM433 | RM230 | 6.4 | 1.46 | 11.0 |
| *qBLAST12* | 12 | | | | | | *RM5746–RM277* | *RM5746* | *20.7* | *0.79* | *31.5* |
| *Leaf blast race IB49* | | | | | | | | | | | |
| *qBLAST8* | 8 | *RM1148–RM72* | *RM72* | *24.8* | *0.33* | *36.5* | *RM72–RM23000* | *RM72* | *13.3* | *0.54* | 21.5 |
| *qBLAST12* | 12 | | | | | | *RM5746–RM277* | *RM5746* | *11.4* | *0.59* | *18.8* |

The underlined QTLs had LOD scores above the permuted LOD thresholds ($\alpha = 0.05$). The QTLs in italics were found across populations and/or years and above LOD threshold in at least one trial

[a] QTLs are declared based on multiple interval mapping with the QGene software (Joehanes and Nelson 2008)

[b] Since disease ratings are scored with lower values indicating enhanced resistance, a positive value for the additive effect of the disease ratings indicates the *O. nivara* parent is the source of the resistance allele. A positive additive effect for the other traits indicates the Bengal allele increases the phenotype

[c] PVE is the percent phenotypic variation explained by an individual QTL. We used $R^2$ values from MIM to represent this

[where sheath blight response is presumably not affected by days to heading, plant height, or plant type (Jia et al. 2007)] clearly indicates the presence of a locus affecting sheath blight resistance in this chromosomal region. LOD peaks at two additional ShB-QTL, *qShB1* and *qShB3*, were found across both populations in both years but the LOD scores were only above the permuted LOD thresholds in the 2008 Wild-2 trial. Resistance was attributed to the *O. nivara* parent when the LOD score was above the permuted LOD threshold but varied when below the threshold. The other significant ShB-QTL, *qShB7* and *qShB11*, were only above the permuted LOD identified in the 2008 Wild-2 trial, with the resistance attributed to Bengal. Across trials, a subthreshold LOD peak at *qShB7* was observed in the 2009 Wild-2 trial while

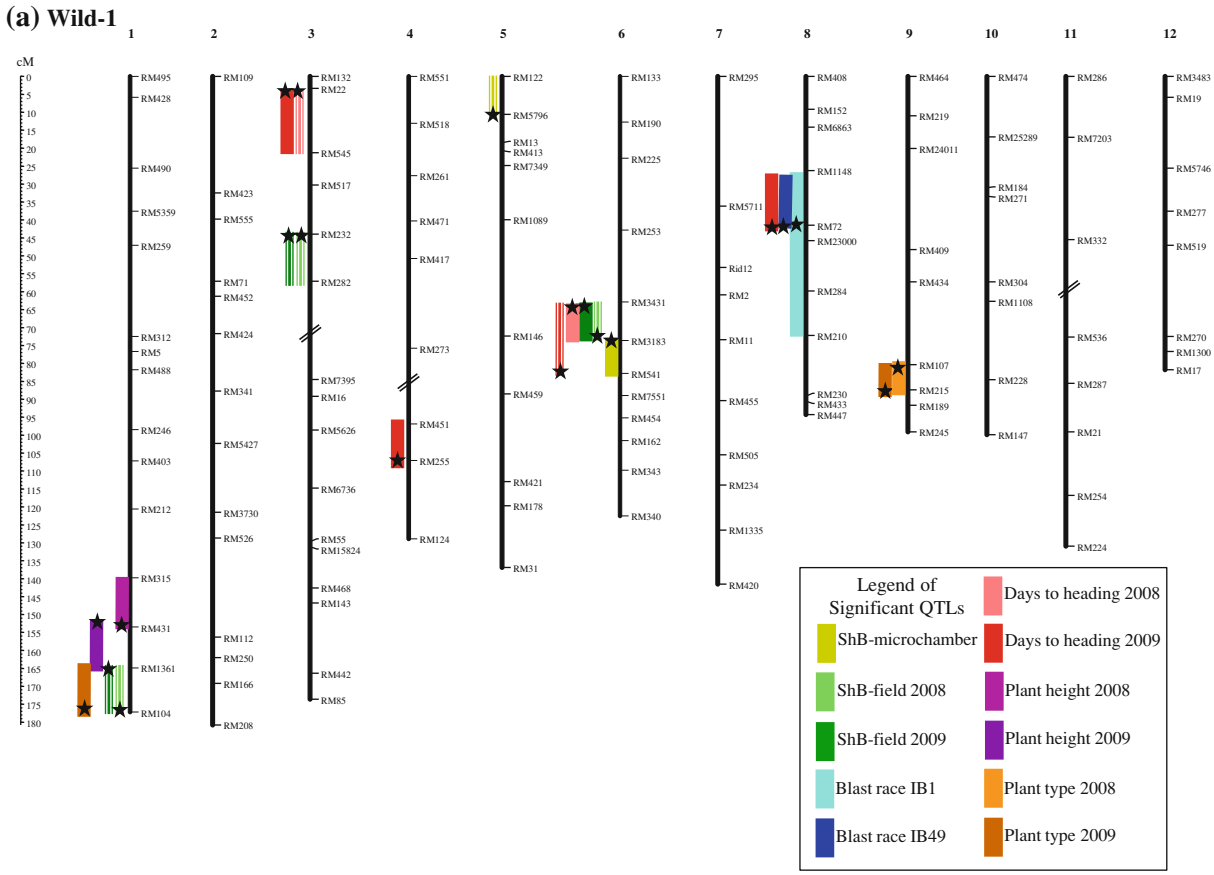


**Fig. 3** Linkage maps in cM (Kosambi 1944) based on 131 SSR markers for the **a** Wild-1 and **b** Wild-2 populations showing QTL for sheath blight in the greenhouse (microchamber) and in the field during 2008 and 2009 along with the confounding traits days to heading, plant height and plant type. QTL for leaf blast races IB1 and IB49 also were identified. *Compound lines* are used for LOD scores below the permuted LOD thresholds ($\alpha = 0.05$). The *diagonal double lines* are *breaks* in the linkage map [The maps were created with MapChart 2.1 (Voorrips 2002).]

evidence in support of *qShB11* was not found in other trials.

## QTL for days to heading, plant height and plant type

LOD peaks for two days to heading (DH) QTL, *qDH3* and *qDH6*, were found in all four trials and exceeded the permutated LOD threshold in two trials. Two additional DH-QTL, *qDH4* and *qDH8*, were identified as above the permutated LOD threshold in the 2009 Wild-1 trial but not supported in any of the other trials. The increased number of days to heading was attributed to the *O. nivara* parent for all the DH-QTL.

One major plant height (PH) QTL, *qPH1*, had LODs that exceeded the permutated LOD threshold for both Wild-1 and Wild-2 and in both years. A second PH-QTL, *qPH12*, was found in Wild-2 with a LOD score above the permutated LOD threshold in the 2008 trial but it was below the threshold in 2009. The increase in plant height was attributed to the *O. nivara* parent in all cases.

Evidence of a major QTL for plant type (PT), *qPT9*, was found in all four trials with LOD scores exceeding the permutated LOD threshold in all but the 2008 Wild-2 trial. The increase in tiller angle reflected by the plant type was attributed to the *O. nivara* parent in both 2008 trials. The additive effect was toward the Bengal parent in both 2009 trials but the values were

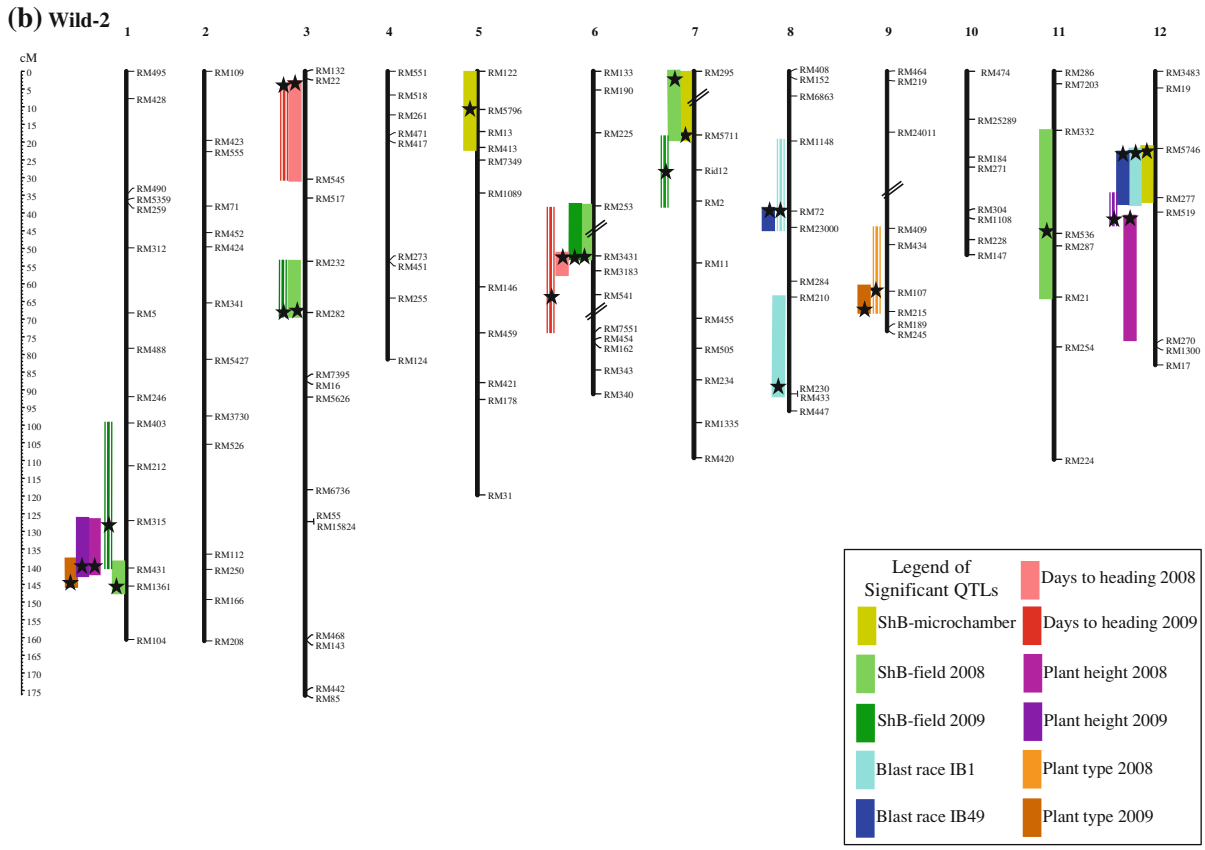


**Fig. 3** continued

very low, 0.04 and 0.01, for Wild-1 and Wild-2 respectively. A second PT-QTL, *qPT1*, was identified in both 2009 trials and this increase in tiller angle was attributed to the *O. nivara* parent in both trials.

QTL for reaction to leaf blast disease

A survey of the BLAST-QTLs across Wild-1 and Wild-2 for the two blast races identified LOD peaks at *qBLAST8-1* in all four tests, with LOD scores above the permuted LOD threshold in three of the tests. The *qBLAST8-1* was below the LOD threshold for the IB1 test of Wild-2 but a second BLAST-QTL, *qBLAST8-2* was only identified in this trial with a LOD above the threshold level. Also in Wild-2 for both blast races, *qBLAST12* was identified with a LOD score above the permuted LOD threshold. No other BLAST-QTLs were identified with LOD scores above the threshold. All resistance associated with these BLAST-QTLs was attributed to the *O. nivara* parent.

## Discussion

Segregation distortion

Most of the distorted markers were randomly distributed across the genome. A moderate level of segregation distortion toward the Bengal parent may be due to discarding some of the sterility genes residing in the *O. nivara* genome. Regions of distorted segregation have been identified in various mapping populations derived from interspecific and intraspecific crosses of several crops (summary in Xu et al. 1997), including rice (Xiao et al. 1996; Huang et al. 1997; Moncada et al. 2001; Septiningsih et al. 2003; Thomson et al. 2003, Zhao et al. 2006a, Liu et al. 2008). The segregation distortion seen on chromosomes 3 and 6 in the KBNT*lpa* × Zhe733 F10-11 (Liu et al. 2008) corresponded to many of the same markers skewed on chromosome 3 in Wild-2 and chromosome 6 in Wild-1 and Wild-2. Two chromosome 6 markers, RM541 and

RM454, which were skewed in both Wild-1 and Wild-2, were reported in the region of the gametophytic and sterility genes (*ga*-1, *S*-5, *S*-6, *S*-8) by Zhao et al. (2006a) in the Nipponbare/Guangluai-4 F2 population. In addition, segregation distortion was found on chromosome 3 in approximately the same region in both Wild-2 and the Nipponbare/Guangluai-4 population in the proximity of the genes *s*-e-1, *S*-9 and *ga*-14 (Zhao et al. 2006a). The large chromosomal regions of segregation distortion noted in Wild-1 or Wild-2 were also found in at least one of the six populations studied by Xu et al. (1997) using RFLP markers. The amount of marker distortion found in the Wild-1 and Wild-2 populations agrees with that noted in other *O. sativa*/*Oryza* species advanced backcross populations (Moncada et al. 2001; Septiningsih et al. 2003; Thomson et al. 2003). Because we did not impose any selection while developing the populations, other than discarding the most sterile BC1, and the chromosome 6 region is common to both populations, we believe the distorted marker segregations may be associated with genes related to sterility.

The fact that at least one whole chromosome was identified as heterozygous in a majority of the progeny lines suggests that there is reduced recombination in these BC2F1 progeny. Genotypic data from other *O. sativa*/*Oryza* species BC2F1 and BC3F1 progeny lines shows a noticeable decrease in whole chromosomes being heterozygous in the BC3F1 progeny (unpublished data).

## Validation of sheath blight QTL among the present and previous studies

Comparing the ShB-QTL identified from our microchamber and field inoculations, only two ShB-QTL, *qShB6-mc* and *qShB7-mc* identified in Wild-1 and Wild-2, respectively, were confirmed in the field studies, with *qShB6* being identified in all four field trials and *qShB7* being identified in all tests of Wild-2. An association mapping study of 217 *O. sativa* accessions found 10 markers associated with sheath blight disease using the microchamber method (Jia Y et al. 2012). Unfortunately, none of the regions reported therein as containing ShB-QTL were confirmed in our study.

The major ShB-QTL, *qShB6*, found in all Wild-1 and Wild-2 field trials, mapped to a location near a major DH-QTL, *qDH6*, with both the resistance and increased flowering time attributed to the *O. nivara* parent. Pinson et al. (2005) also identified both a ShB-QTL and a DH-QTL in the same region in the Lemont/TeQing population, suggesting that delayed flowering time may be confounding disease development in their field study. It is especially interesting, therefore, that *qShB6* was found to be significant in the present microchamber study (*qShB6-mc*), where plants escaping disease development by delaying heading until field conditions are too cool for the pathogen to develop is not a factor. The *Hd1* QTL (*Se1* gene) identified by Yano et al. (2000) in a Nipponbare/Kasalath population using map-based cloning is also known to reside in this region containing *qShB6* and *qDH6*. Since we observed photoperiod sensitivity in both *O. nivara* parents (Table 1), this may be at least one of the genes controlling the sensitivity, especially since Yano et al. (2000) suggest that *Hd1* promotes heading under short-day conditions and inhibits it under long-day conditions (Lin et al. 2000).

The *qShB7* identified in all three Wild-2 trials was in the same region as a SB-QTL identified in field studies of Lemont/TeQing (Pinson et al. 2005), Lemont/Jasmine 85 (Pan et al. 1999; Liu et al. 2013) and HP2216/Tetep (Channamallikarjuna et al. 2010) populations. It should also be noted that a gene conferring resistance to sheath blight toxin (Costanzo et al. 2011) was mapped close to RM11 on chromosome 7, below *qShB7* found in this study. Further research would be required to determine whether the *O. nivara* alleles identified in the present study are the same as or different from the alleles reported in these other studies.

A minor ShB-QTL, *qShB5-mc*, was found only with the microchamber and was attributed to the Bengal parent, which is known to be moderately resistant to sheath blight disease. In the Lemont/Jasmine85 population (Liu et al. 2009), a ShB-QTL from the microchamber inoculation was also found near *qShB5*. Lastly, *qShB12-mc* agreed with ShB-QTL identified by Sato et al. (2004) and Nelson et al. (2012) in field studies.

A second ShB-QTL not associated with days to heading or plant height, *qShB3*, was identified in all four field tests in the same location as ShB-QTL identified in Lemont/TeQing (Pinson et al. 2005), Lemont/Jasmine85 (Pan et al. 1999) and HP2216/Tetep (Channamallikarjuna et al. 2010), with resistance being associated with the *O. nivara* parent in the

Wild-2 population and the Bengal parent in the Wild-1 population. The LOD score was above the permuted threshold level in Wild-2, suggesting that the allele from this *O. nivara* parent, acc. IRGC104705, is providing a higher level of resistance.

LOD peaks at *qShB1* were observed in all four field trials with the resistance attributed to the Bengal parent in all trials. In addition, the major PH-QTL, *qPH1*, found in all four field trials, was located in this region and the increased plant stature was attributed to the *O. nivara* parent. The *sd1* gene for semi-dwarf plant stature is located in this same region (Asano et al. 2007) and Kim et al. (2009) reported that Bengal carries the *sd1* gene introduced from the rice cultivar Dee-geo-woo-gen. Thus, this ShB-QTL, which was not observed under microchamber conditions, is most likely confounded with an increase in plant stature introduced from *O. nivara*.

Whole genome sequences of 13 rice inbred cultivars, including Bengal, were used to identify non-synonymous SNPs and candidate genes for sheath blight resistance (Silva et al. 2012). Their principal component–biplot display of the variants on chromosome 9 clearly separated Bengal with the susceptible cultivars even though the vector was shorter, most likely due to it being the only medium grain cultivar. Subsequently, SNP alleles were identified for 12 candidate sheath blight resistance genes and the Wild-1 *O. nivara* parent, IRGC100898, produced one resistance allele on chromosome 4. Unfortunately, no ShB-QTL was identified in this study on chromosome 4 to confirm the presence of a sheath blight resistance gene contributed by the *O. nivara* parent.

In Wild-2 the *qPH12* was in the location of a plant height QTL in the Jefferson/*O. rufipogon* (Thomson et al. 2003) and IR64/Azucena (Li et al. 2003) populations. To date, we are not aware of any genes associated with plant stature that have been fine-mapped or cloned in this region.

The most significant QTL for plant type, *qPT9*, identified as tiller angle in other studies (Chen et al. 2008; Li et al. 1999; Wang et al. 2011), has been cloned as the tiller angle control-1 (*TAC-1*) gene (Wang and Li 2008; Yu et al. 2007). The more spreading plant type, in other words increased tiller angle, was attributed to the *O. nivara* parent. A minor QTL, *qPT1*, identified in the second year in both Wild-1 and Wild-2 was in the same region as a QTL identified in the early Lemont/TeQing population (Li et al. 1999). Even though a significant correlation was not identified between tiller angle (plant type) and sheath blight disease reaction in the field in this study, it is a trait that could influence the disease reaction, especially in field inoculations. We identified significant differences in tiller angle mapped to this region, but effects, if any, on sheath blight response were so small that not even a subthreshold LOD peak was identified for this region.

### Validation of leaf blast QTLs in previous studies

All the QTL presently identified for leaf blast mapped to regions of known blast genes (Koide et al. 2009) with the most significant across years and populations being *qBLAST8-1* in the region of *Pi11*, *Pi29* and *Pi33*. A blast resistance QTL was also reported here by Jia and Liu (2011). The *qBLAST12* found in Wild-2 resides in the region of blast genes *Pita*, *Pi4* and *Pi24* and *qBLAST8-2*, identified in 2009 Wild-1, is in the region of *Pizh* (Liu et al. 2010).

A survey of the genotypes for the Wild-1 and Wild-2 progeny lines that were the most resistant to both blast races revealed that the region of RM72 on chromosome 8 and/or the region near RM5736 on chromosome 12 was heterozygous in all but one line resistant to both races. It is not uncommon for blast resistance genes to confer resistance to multiple races. Further investigation of these resistant lines would be required to determine if the gene and/or alleles introgressed from the *O. nivara* parent are novel or are the same as those reported elsewhere.

## Conclusion

To our knowledge, this is the first report of the AB-QTL strategy being used to map sheath blight QTL with a wild *Oryza* species (*O. nivara*) accession as the donor parent. Previously, the AB-QTL strategy was used to map QTL for grain yield, yield-contributing traits and grain quality parameters (McCouch et al. 2007; Brondani et al. 2002; Jing et al. 2010; Tan et al. 2008; Wickneswari et al. 2012). In addition, this is only the second sheath blight QTL study to document QTL for tiller angle (plant type). Tiller angle was documented in the Lemont/Teqing population but reported separately (Li et al. 1999; Pinson et al. 2005). Even in cases where the *O. nivara* QTL mapped to

previously reported gene(s) or QTL sites, it is possible that the alleles contained in these *O. nivara* parents, selected by nature over eons under weedy conditions, are distinct from those identified from highly selected, cultivated parents. Fine mapping of the validated sheath blight (*qShB3*, *qShB6*) and blast (*qBLAST8-1*, *qBLAST12*) QTL could lead to identification of novel resistance alleles useful to breeders and easily be incorporated into elite breeding lines with additional backcrossing. Both the Wild-1 and Wild-2 populations have been advanced to the BC2F5 generation to develop backcross inbred line (BIL) populations so that the identified QTL can be further evaluated in a more homozygous background. Because the *O. nivara* segments are anticipated to be smaller and fewer per progeny line among the BILs, location and gene effect estimates should be more accurate, and the BILs can serve as improved germplasm for incorporating the desired alleles into elite germplasm with less linkage drag.

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## References

Ali ML, Sanchez PL, Yu S, Lorieux M, Eizenga GC (2010) Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*). Rice 3:218–234

Amante AD, de la Pena R, Sitch LA, Leung H, Mew TW (1990) Sheath blight (ShB) resistance in wild rices. Int Rice Res Newsl 15:5

Asano K, Takashi T, Miura K, Qian Q, Kitano H, Matsuoka M, Ashikari M (2007) Genetic and molecular analysis of utility of *sd1* alleles in rice breeding. Breed Sci 57:53–58

Atkins JG, Robert AL, Adair CR, Goto K, Kozaka T, Yanagida R, Yamada M, Matsumoto S (1967) An international set of rice varieties for differentiating races of "*Pyricularia oryzae*". Phytopathology 57:297–301

Bernacchi D, Beck BT, Eshed Y, Lopez Y, Petiard V, Uhlig J, Zamir D, Tanksley SD (1998) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Berruyer R, Adreit H, Milazzo J, Gaillard S, Berger A, Dioh W, Lebrun MH, Tharreau D (2003) Identification and fine mapping of *Pi33*, the rice resistance gene corresponding to the *Magnaporthe grisea* avirulence gene ACE1. Theor Appl Genet 107:1139–1147

Brar DS, Khush GS (1997) Alien introgression in rice. Plant Mol Biol 35:35–47

Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theor Appl Genet 104:1192–1203

Channamallikarjuna V, Sonah H, Prasad M, Rao JN, Chand S, Upreti HC, Singh NK, Sharma TR (2010) Identification of major quantitative trait loci *qSBR11-1* for sheath blight resistance in rice. Mol Breed 25:155–166

Chen ZX, Zou JH, Xu JY, Tong YH, Tang SZ, Wang ZB, Jing RM, Ling B, Tang J, Pan XB (2000) A preliminary study on resources of resistance to rice sheath blight. Chin J Rice Sci 14:15–18

Chen P, Jiang L, Yua C, Zhang W, Wang J, Wan J (2008) The identification and mapping of a tiller angle QTL on rice chromosome 9. Crop Sci 48:1799–1806

Correll JC, Boza EJ, Seyran E, Cartwright RD, Jia Y, Lee FN (2009) Examinations of the rice blast pathogen population diversity in Arkansas, USA-stable or unstable? In: Wang GL, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, Dordrecht, pp 217–228

Costanzo S, Jackson AK, Brooks SA (2011) High-resolution mapping of *Rsn1*, a locus controlling sensitivity of rice to a necrosis-inducing phytotoxin from *Rhizoctonia solani* AG1-IA. Theor Appl Genet 123:33–41

Couch BC, Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. Mycologia 94:683–693

Eizenga GC, Lee FN, Jia Y (2002) Use of DNA markers to identify blast resistance genes in the wild relatives of rice (*Oryza* sp.). In: Norman RJ, Meullenet J-F (eds) BR Wells Rice Research Studies 2001. University of Arkansas Agricultural Experiment Station Research Series 495:19–23

Eizenga GC, Agrama HA, Lee FN, Jia Y (2009) Exploring genetic diversity and potential novel disease resistance genes in a collection of rice wild relatives. Genet Resour Crop Evol 56:65–76

Fu D, Chen L, Yu G, Liu Y, Lou Q, Mei H, Xiong L, Li M, Xu X, Luo L (2011) QTL mapping of sheath blight resistance in a deep-water rice cultivar. Euphytica 180:209–218

Huang N, Parco A, Mew T, Magapantay G, McCouch S, Guiderdoni E, Xu J, Subudhi P, Angelos ER, Khush GS (1997) RFLP mapping of isozymes, RAPD and QTLs for

grain shape, brown planthopper resistance in a doubled haploid rice population. Mol Breed 3:105–113
IRRI (2002) Standard evaluation system for rice (SES). International Rice Research Institute, Manila, p 33
Jena KK, Khush GS (2000) Exploitation of alien species in rice improvement—opportunities, achievements and future challenges. In: Nanda JS (ed) Rice breeding and genetics: research priorities and challenges. Science Publishers, Inc., Endfield, pp 271–285
Jeung JU, Kim BR, Cho YC, Han SS, Moon HP, Lee YT, Jena KK (2007) A novel gene, *Pi40(t)*, linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. Theor Appl Genet 115:1163–1177
Jia Y, Liu G (2011) Mapping quantitative trait loci for resistance to rice blast. Phytopathology 101:176–181
Jia Y, Correa-Victoria F, McClung A, Zhu L, Liu G, Wamishe Y, Xie J, Marchetti MA, Pinson SRM, Rutger JN, Correll JC (2007) Rapid determination of rice cultivar response to the sheath blight pathogen *Rhizoctonia solani* using a microchamber screening method. Plant Dis 91:485–489
Jia Y, Liu G, Costanzo S, Lee S, Dai Y (2009) Current progress on genetic interactions of rice with rice blast and sheath blight fungi. Front Agric China 3(3):231–239
Jia L, Yan W, Zhu C, Agrama HA, Jackson A, Yeater K, Li X, Huang B, Hu B, McClung A, Wu D (2012) Allelic analysis of sheath blight resistance with association mapping in rice. PLoS ONE 7(3):e32703. doi:10.1371/journal.pone.0032703
Jia Y, Liu G, Correa-Victoria FJ, McClung AM, Oard JH, Bryant RJ, Jia MH, Correll JC (2012) Registration of four rice germplasm lines with improved resistance to sheath blight and blast diseases. J Plant Reg 6:95–100
Jing Z, Qu Y, Chen Y, Pan D, Fan Z, Chen J, Li C (2010) QTL analysis of yield-related traits using an advanced backcross population derived from common wild rice (*Oryza rufipogon* L.). Mol Plant Breed 1(1):1–10
Joehanes R, Nelson JC (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 23:2788–2789
Khush GS, Jena KK (2009) Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: Wang GL, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, Dordrecht, pp 1–10
Khush GS, Balacangco E, Ogawa T (1990) A new gene for resistance to bacterial blight from *Oryza longistaminata*. Rice Genet Newsl 7:121–122
Kim SI, McKenzie KS, Tai TH (2009) A molecular survey of SD1 alleles used in rice cultivars. SABRAO J Breed Genet 41:25–40
Koide Y, Kobayashi N, Xu D, Fukuta Y (2009) Resistance genes and selection DNA markers for blast disease in rice (*Oryza sativa* L.). JARQ 43:255–280. (Japan Agricultural Research Quarterly http://www.jircas.affrc.go.jp)
Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175
Lakshmanan P (1991) Resistance to sheath blight (ShB) and brown spot (BS) in lines derived from *Oryza officinalis*. Int Rice Res Newsl 16:8
Lee FN, Rush MC (1983) Rice sheath blight: a major rice disease. Plant Dis 67:829–832
Lee FN, Dilday RH, Moldenhauer K, Rutger JN, Yan W (1999) Sheath blight and rice blast resistance in recently introduced rice germplasm. In: Norman RJ, Johnston TH (eds) BR Wells Rice Research Studies 1998, University of Arkansas Agricultural Experiment Station Research Series 468:195–210
Li ZK, Paterson AH, Pinson SRM, Stansel JW (1999) RFLP facilitated analysis of tiller and leaf angles in rice (*Oryza sativa* L.). Euphytica 109:79–84
Li H, Song CY, Cong WB, Wang GL (2000) Evaluation and screening of resistance in key rice varieties to sheath blight. Plant Protect 26:19–21
Li ZK, Yu SB, Lafitte HR, Huang N, Courtois B, Hittalmani S, Vijayakumar CHM, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S, Khush GS (2003) QTL x environmental interactions in rice. I. Heading date and plant height. Theor Appl Genet 108:141–153
Lin HX, Yamamoto T, Sasaki T, Yano M (2000) Characterization and detection of epistatic interactions of 3 QTLs, *Hd1*, *Hd2*, and *Hd3*, controlling heading date in rice using nearly isogenic lines. Theor Appl Genet 101:1021–1028
Linscombe SD, Jodari F, McKenzie KS, Bollich PK, White LM, Groth DE, Dunand RT (1993) Registration of Bengal rice. Crop Sci 33:645
Liu G, Bernhardt JL, Jia MH, Wamishe YA, Jia Y (2008) Molecular characterization of the recombinant inbred line population derived from a *japonica-indica* rice cross. Euphytica 159:73–82
Liu G, Jia Y, Correa-Victoria FJ, Prado GA, Yeater KM, McClung A, Correll JC (2009) Mapping quantitative trait loci responsible for resistance to sheath blight in rice. Phytopathology 99:1078–1084
Liu J, Wang X, Mitchell T, Hu Y, Liu X, Dai L, Wang GL (2010) Recent progress and understanding of the molecular mechanisms of the rice–*Magnaporthe oryza*e interaction. Mol Plant Pathol 11:419–427
Liu G, Jia Y, McClung A, Oard JH, Lee FN, Correll JC (2013) Confirming QTLs and finding additional loci responsible for resistance to rice sheath blight disease. Plant Dis 97:113–117
Manosalva PM, Davidson RM, Liu B, Zhu X, Hulbert SH, Leung H, Leach JE (2009) A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. Plant Physiol 149:286–296
Marchetti MA, Bollich CN (1991) Quantification of the relationship between sheath blight severity and yield loss in rice. Plant Dis 75:773–775
McCouch SR, Sweeney M, Li J, Jiang H, Thomson M, Septiningsih E, Edwards J, Moncada P, Xiao J, Garris A, Tai T, Martinez C, Tohme J, Sugiono M, McClung A, Yuan LP, Ahn SN (2007) Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*. Euphytica 154:317–339
Meena B, Ramamoorthy V, Banu JG, Thangavelu R, Muthusamy M (2000) Screening of rice genotypes against sheath blight disease. J Ecobiol 12:103–109
Moncada P, Martínez CP, Borrero J, Chatel M, Gauch H Jr, Guimaraes E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza*

*sativa* × *Oryza rufipogon* $BC_2F_2$ population evaluated in an upland environment. Theor Appl Genet 102:41–52

Nelson JC, Oard JH, Groth D, Utomo HS, Jia Y, Liu G, Moldenhauer KAK, Correa-Victoria FJ, Fjellstrom RG, Scheffler B, Prado GA (2012) Sheath-blight resistance QTLs in *Japonica* rice germplasm. Euphytica 184:23–34

Pan XB, Rush MC, Sha XY, Xie QJ, Linscombe SD, Stetina SR, Oard JH (1999) Major gene, nonallelic sheath blight resistance from the rice cultivars Jasmine 85 and TeQing. Crop Sci 39:338–346

Pinson SRM, Capdevielle FM, Oard JH (2005) Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. Crop Sci 45:503–510

Pinson SRM, Oard JH, Groth D, Miller R, Marchetti MA, Shank AR, Jia MH, Jia Y, Fjellstrom RG, Li Z (2008) Registration of TIL:455, TIL:514, and TIL:642, three rice germplasm lines containing introgressed sheath blight resistance alleles. J Plant Reg 2:251–254

Prasad B, Eizenga GC (2008) Rice sheath blight disease resistance identified in *Oryza* spp. accessions. Plant Dis 92:1503–1509

Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang GL (2006) The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site–leucine-rich repeat protein and is a member of a multigene family in rice. Genetics 172:1901–1914

Raina GL, Sidhu JS, Bharaj TS, Gautam RK, Om V, Vir O (1999) Multiple disease resistance in rice. Crop Improv 26:63–65

Rush MC, Lindberg GD (1996) Rice disease research. Rice J 77:49–52

Sato H, Ideta O, Ando I, Kunihiro Y, Hirabayashi H, Iwano M, Miyasaka A, Nemoto H, Imbe T (2004) Mapping QTL for sheath blight resistance in the rice line WSS2. Breed Sci 54:265–271

Septiningsih EM, Prasetiyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor Appl Genet 107:1419–1432

Sharma A, McClung AM, Pinson SRM, Kepiro JL, Shank AR, Tabien RE, Fjellstrom R (2009) Genetic mapping of sheath blight resistance QTLs within tropical *japonica* rice cultivars. Crop Sci 49:256–264

Silva J, Scheffler B, Sanabria Y, De Guzman C, Galam D, Farmer A, Woodward J, May G, Oard J (2012) Identification of candidate genes in rice for resistance to sheath blight disease by whole genome sequencing. Theor Appl Genet 124:63–74

Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease. Trends Biotechnol 27:141–150

Tai T, Tanksley SD (1990) A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol Biol Rep 8:297–303

Tan L, Zhang P, Liu F, Wang G, Ye S, Zhu Z, Fu Y, Cai H, Sun C (2008) Quantitative trait loci underlying domestication- and yield-related traits in an *Oryza sativa* × *Oryza rufipogon* advanced backcross population. Genome 51:692–704

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Tharreau D, Fudal I, Andriantsialona D, Utami SD, Fournier E, Lebrun M-H, Notteghem J-L (2009) World population structure and migration of the rice blast fungus, *Magnaporthe oryzae*. In: Wang GL, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, Dordrecht, pp 209–215

Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinez CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

Van Berloo R (2008) GGT 2.0: versatile software for visualization and analysis of genetic data. J Hered 99:232–236

Van Ooijen JW (2006) JoinMap 4. Software for genetic linkage maps in experimental populations. Kayazma BV, Wageningen

Venu RC, Jia Y, Gowda M, Jia MH, Jantasuriyarat C, Stahlberg E, Li H, Rhineheart A, Boddhireddy P, Singh P, Rutger N, Kudrna D, Wing R, Nelson JC, Wang GL (2007) RL-SAGE and microarray analysis of the rice transcriptome after *Rhizoctonia solani* infection. Mol Genet Genomics 278:421–431

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wamishe YA, Jia Y, Singh P, Cartwright RD (2007) Identification of field isolates of *Rhizoctonia solani* to detect quantitative resistance in rice under greenhouse conditions. Front Agric China 1:361–367

Wang Y, Li J (2008) Rice, rising. Nat Genet 40:1273–1275

Wang L, Wang A, Huang X, Zhao Q, Dong G, Qian Q, Sang T, Han B (2011) Mapping 49 quantitative trait loci at high resolution through sequencing-based genotyping of rice recombinant inbred lines. Theor Appl Genet 122:327–340

Wickneswari R, Bhuiyan MAR, Kalluvettankuzhy KS, Lim LS, Thomson MJ, Md. Kairudin Narimah MK, Md. Zain Abdullah MZ (2012) Identification and validation of quantitative trait loci for agronomic traits in advanced backcross breeding lines derived from *Oryza rufipogon* × *Oryza sativa* cultivar MR219. Plant Mol Biol Rep 30:929–939

Xiao J, Li J, Yuan L, Tanksley SD (1996) Identification of QTLs affecting traits of agronomic importance in recombinant inbred population derived from a sub-specific rice cross. Theor Appl Genet 92:230–244

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in $F_2$, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet 253:535–545

Xu Q, Yuan XP, Yu HY, Wang YP, Tang SX, Wei XH (2011) Mapping quantitative trait loci for sheath blight resistance in rice using double haploid population. Plant Breed 130:404–406

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12:2473–2483

Yu B, Lin Z, Li H, Li X, Li J, Wang Y, Zhang X, Zhu Z, Zhai W, Wang X, Xie D, Sun C (2007) *TAC1*, a major quantitative trait locus controlling tiller angle in rice. Plant J 52:891–898

Zhao B, Deng Q, Zhang Q, Li J, Ye S, Liang Y, Peng Y, Li P (2006a) Analysis of segregation distortion of molecular markers in $F_2$ population of rice. Acta Genet Sin 33:449–457

Zhao M, Zhang Z, Zhang S, Li W, Jeffers DP, Rong T, Pan G (2006b) Quantitative trait loci for resistance to banded leaf and sheath blight in maize. Crop Sci 46:1039–1045